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COMPARATIVE EFFECT OF PENICILLIN AND SUL-
FONAMIDE DRUGS ON THE IMMUNE RESPONSE
OF RABBITS TO PNEUMOCOCCUS INFECTION
AND THE RELATION OF IMMUNITY TO
BACTERIAL CHEMOTHERAPY

A DISSERTATION SUBMITTED TO THE FACULTY OF THE DIVISION OF THE BIOLOGICAL
SCIENCES IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF BACTERIOLOGY AND PARASITOLOGY
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INTRODUCTION

The development of clinically apparent infectious disease and the outcome of the established infection are dependent upon 2 variables, the pathogenicity of the microorganism and the immunity, both natural and acquired, of the host. A third factor is introduced with the use of chemotherapeutic drugs. By definition a therapeutic substance is one which influences the infection favorably from the point of view of the host, a result attained as a consequence of its action on either the parasite or host. Thus, the therapeutic use of antiserum reinforces the immunity of the host, affecting the parasite only indirectly. On the other hand, the chemotherapeutic drugs appear to function primarily through their direct action on the parasite. The ideal chemotherapeutic agent would show such sharp specificity of action that it would be tolerated by the host in concentrations sufficient to

sterilize the body of parasites. This ideal has not yet been attained and the drugs effective in bacterial infections, compounds of the sulfonamide series and the antibiotic penicillin, are characterized by a bacteriostatic action or inhibition of cell division of the invading microorganisms. Such action is clearly not sufficient to eliminate the infection, and the efficacy of the drug is dependent upon the development of an effective immune response on the part of the host. It would appear, therefore, that tolerance of an effective chemotherapeutic agent must include a relatively uninhibited immune response, or possibly even an acceleration of it, effects which may be produced either directly or indirectly by the drug. Thus the interrelationships existing between host, parasite, and drug may become exceedingly complex.

The dependence of the action of the drug on the cooperation of the host is indicated in a variety of ways. For example, vitamin deficiency (Robertson, 1934; Cannon, 1940) and inadequate protein reserves (Cannon, et al., 1943; Mills and Cottingham, 1943), as they decrease the functional activity of the defense mechanism, influence the success and progress of chemotherapy. Bacterial infections which are characterized especially by their debilitating effects require optimum chemotherapeutic action for their control. Local cellular and humoral activity (Robertson, 1938) and products of the inflammatory reaction (Menkin, 1945) in conjunction with

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drug may be sufficient to eradicate the infection in the absence of acquired immunity. On the other hand, however, the inflammatory exudate may inhibit sulfonamide activity (Lockwood, 1938; MacLeod, 1940; Boroff, et al., 1942), and the antibody-neutralizing effect of infected exudates (Cole, 1917; Felton and Bailey, 1926; Boivin and Delaunay, 1945) may prevent effective cooperation of the immune mechanism. A drug that functions independently of these factors provides more efficient chemotherapeutic action.

In well established infections the elaboration of specific immune substances is necessary for recovery (Bull, 1915; Wright, 1927; Robertson, et al., 1928). Within certain limits the extent to which the immune response contributes to the recovery process is reflected by the amount of detectable antibody present during the acute phase of an infection; failure to demonstrate immune substances, or their presence in only low titer, suggests that they may be used up in *in vivo* antigen-antibody reactions associated with the immune response (cf. Blake, 1918; Park and Cooper, 1928; Tillett, 1942). Under ideal conditions of chemotherapy, the response is such that the development of immunity proceeds in a manner similar to that which occurs when killed antigen is injected (cf. Wright, 1927; Reichel, 1939).

Factors that condition pathogenicity influence the outcome of chemotherapeutic action. Natural infections probably are most often initiated by a relatively small inoculum. If therapy is begun early bacteriostasis in conjunction with natural immunity may be sufficient to eliminate the infection almost immediately. Delay in initiating therapy increases the difficulty of eliminating the parasite not only because the infecting organism has had time to in-

crease in numbers and to elaborate its toxic products but also because an opportunity is provided for the invading bacteria to establish themselves in small abscesses and other foci in which they are partially protected from the action of both antibody and chemotherapeutic drug (cf. Holman and Duff, 1938; Garrod and Ungar, 1944; Chain and Duthie, 1945). On the other hand, if the drug is sufficiently active and therapy is initiated early multiplication of the organisms may be prevented to the extent that antigenic stimulus is abolished and no acquired immunity develops.

The elaboration of toxins, and other products of bacterial activity, influence the outcome of chemotherapy since the scope of antitoxic activity of chemotherapeutic agents in present day use appears to be limited (Huntington, 1938; Neter, 1939; Zahl, et al., 1944; Neter and Will, 1944). Although Boor and Miller (1945, 1946) recently demonstrated that large doses of penicillin have a synergistic neutralizing action on endotoxins of meningococci and gonococci, it is possible that under ordinary conditions of chemotherapy the soluble products of bacterial activity are affected only indirectly insofar as the drugs are able to prevent their elaboration by inhibiting bacterial growth. By this action, however, the amount of living antigen or its soluble products is reduced; a quantitative alteration in the antigen-antibody relationship results which in effect amounts to a relative increase in antibodies. It is possible that in certain types of infections there may be considerable indirect benefit to the general over all immune mechanism of the host derived from chemotherapy in this way.

The extent to which the host's defenses or the virulence of the infecting microorganism influences the efficacy of chemotherapy depends primarily on the

activity of the drug. A drug to which the infecting microorganism is relatively insusceptible, either by natural or acquired resistance, is dependent to a great extent on the host's defenses for successful chemotherapeutic response. While bacteriostasis alone may account for part of the chemotherapeutic action of a drug, the cooperation of the defense mechanism of the host is required for complete elimination of the infecting organism. The ideal chemotherapeutic agent is one which will destroy the susceptible parasite independently of the host's defenses.

It is ordinarily assumed that effective response to the therapeutic administration of sulfonamide drugs is dual in nature, the drug exerting a bacteriostatic effect while the invading microorganisms are destroyed by the host through natural or acquired immune mechanisms (Marshall, 1940). The phagocytic activity of the cells (Domagk, 1937; Levaditi and Vaisman, 1935; Long and Bliss, 1937; Gay and Clark, 1937) in the presence of antibody, produced either by passive or active immunization or acquired during the course of an infection, increases the curative effects of sulfonamide chemotherapy (Mellon and Bambas, 1937; Powell and Jamieson, 1939; Fleming, 1939; McLean, et al., 1939; Wood and Long, 1939; Gregg, et al., 1940).

The degree of bacteriostasis, and the speed at which the bacteriostatic activity of a drug becomes functional, influence the outcome of chemotherapeutic action. Sulfonamide bacteriostasis usually is incomplete and operates after a lag of 4-6 hours (Whitby, 1938; McIntosh and Whitby, 1939; Spring, et al., 1940; Lowell, et al., 1941).

The early bacteriological cures observed in penicillin therapy of gonococcal infections (Mahoney, et al., 1943; Cohn and Kornblith, 1945; Long, 1945).

the rapid disappearance of spirochetes from the primary lesions of syphilis (Mahoney, et al., 1943; Moore, et al., 1944), and the early response to penicillin therapy of other types of infections (Keefer, et al., 1943; Officer, et al., 1944; Tillett, Cambier and McCormack, 1944; Mead, Harris and Finland, 1945) suggest that the chemotherapeutic activity of this drug is relatively independent of an immune mechanism. MacLeod and Stone (1945) recently reported the results of an experimental study which appear to confirm this hypothesis.

It would appear, however, that one cannot minimize the importance of an immune mechanism to penicillin therapy solely on the basis of early bacteriological cures, either in clinical or experimental studies. In the usual clinical infections the microorganisms have opportunity during the incubation period to provoke an immune response. In experimental infections the inoculum is usually sufficiently large to induce an immune response very early, and in the presence of adequate bacteriostasis immunity may become functional within a few hours.

In vitro studies (Fleming, 1929; Fisher, 1943; Rammelkamp and Keefer, 1943; Hobby and Dawson, 1944; Garrod and Ungar, 1944; Todd, 1945) show that sensitive microorganisms are killed rapidly in the presence of small amounts of penicillin, although in concentrations effective in vivo complete sterility is never obtained (Hobby, Meyer and Chaffee, 1942; Dawson, et al., 1943). The results of in vivo experiments (Hobby, Meyer and Chaffee, 1942; Robinson, 1943) suggest a bactericidal activity, but it is possible that the usual effective in vivo concentrations of penicillin are only bacteriostatic. Under such conditions, however, bacteriostasis appears to be extremely efficient.

Penicillin activity, unlike that of the

sulfonamides, appears to become effective immediately at the time of cell division or on multiplication of the organism (Abraham, et al., 1941), and displays no lag in activity in the presence of actively dividing cells. Also, penicillin appears to be unaffected by infected serums, exudates or transudates (Abraham, et al., 1941).

It is presumably the failure of development of an adequate immune response that accounts for the not uncommon observation that on cessation of administration of chemotherapeutic agents clinical symptoms of the infection reappear. Evidence has been presented by a number of workers which suggests that antibody response is not influenced by the administration of sulfonamide drugs (MacLeod, 1939; McIntosh and Whitby, 1939; Levine, Larson and Bieter, 1939; Tillett, 1942), and is comparable to the events that occur in spontaneous recovery (cf. Robertson, et al., 1928; Goodner, 1928; Sia, Robertson and Woo, 1928).

The development of acquired immunity, as evidenced by antibody response, or other measurable immune response, during penicillin therapy of experimental or clinical infections, appears not to have been studied as yet. Since the therapeutic activity of this drug appears to be much greater than that of the sulfonamide drugs one might expect to find an altogether different type of immune response of the host to infections treated with penicillin as compared with sulfonamide treatment or spontaneous recovery.

Of the complex factors associated with the action of the drug, that of the immune response of the host is one of the most important, if not the most important. This study is devoted to an examination of this aspect of the chemotherapy of experimental pneumococcus infection in the rabbit and attempts to define the

relative importance of immunity and the immune response in the animal undergoing chemotherapy.

MATERIALS AND METHODS

Pneumococcus infection in the rabbit.—White rabbits weighing approximately 2 kg were used. A virulent strain of Type I pneumococcus was inoculated intradermally as described by Goodner (1928). The virulence of the culture was such that 10^{-6} ml of an 8–10 hour Felton's broth culture, representing 10,000 median lethal doses (rabbit), invariably killed untreated animals in 36–48 hours by generalization of the infection.

Drugs used and the method of administration.—Penicillin and sulfapyridine were used in the majority of experiments with pneumococcus infection of the rabbit. Since sulfadiazine and sulfamerazine compare favorably with sulfapyridine in therapeutic effectiveness in pneumococcus infections (Feinstone, et al, 1940; Finland, et al, 1941; Flippin, et al, 1943; Genecin, Austrian and Nelson, 1945; Morgan and Wylie-Smith, 1943) and exhibit less toxic manifestations during their administration (Plummer and Ensworth, 1940; Reinhold, et al, 1941; Long, 1941; Dowling, et al, 1944), these 2 drugs were compared with sulfapyridine. Penicillin was given in 5 intramuscular injections of 500 Oxford units each per day for the 4-hour series and 1,000 Oxford units each for the 24 hour group, and the sulfonamide drugs were administered by stomach tube in 1.0 g doses twice daily. Treatment was initiated at 4 or 24 hours after intradermal injection except for sulfadiazine and sulfamerazine which were initiated at 24 hours only. Therapy was carried out for 4 days except for a few animals in the sulfapyridine group which required the drug for longer periods.

Clinical course of the disease.—Observations were made for 12 days. The dermal lesions were observed daily and the severity estimated by plus signs, from + to represent a tuberculin type lesion to ++++ to represent one of maximal intensity, consisting of edema extending across the midline. The animals were weighed on alternate days and temperatures taken daily.

Blood cultures.—Blood for culture was collected daily until 3 consecutive negative cultures were obtained. Blood from animals receiving sulfonamide drugs was cultured in Felton's broth containing 5 mg para-aminobenzoic acid per 100 ml. No penicillin-inhibitor was used for blood cultures from penicillin-treated rabbits; 12 hours had elapsed after the last dose of the drug and assays performed on blood from these animals revealed no penicillin activity after 6 hours, using this pneumococcus strain as the test organism.

Examination of lesion fluid.—From time to time fluid was aspirated from the lesions for culture, penicillin assay and serological tests.

Antibody titrations.—Approximately 5 ml of blood was collected from the marginal ear veins on alternate days for antibody titrations. Agglutinin titers were determined on 2-fold dilutions of the serum, using a heat-killed, washed, 18 hour broth culture of pneumococci. The agglutinin titer was expressed as the reciprocal of the highest dilution of serum in which agglutination occurred. A modification of the method of Reed and Muench (1938) was used for determining the protective antibody titer. The method employed consisted of titrating 10-fold dilutions of an 8–10 hour culture of pneumococcus against a constant volume of serum. The serum was injected in 0.2 ml volume 1–2 hours before the infecting dose of pneumococci. The mice were observed for 72 hours. The protective antibody titer was expressed as the number of LD₅₀ doses of pneumococci against which 0.1 ml of serum protected. The LD₅₀ dose for mice was less than 10 pneumococci by intraperitoneal inoculation.

Re-infection procedures.—All rabbits that recovered were tested for resistance at the end of the 12 day observation period by the intradermal inoculation of 100,000 median lethal doses of pneumococci; this injection was made into the opposite side from that of the original infecting dose. Severity of lesions and length of survival were the criteria used in estimating the state of resistance of survivors.

Immunization of rabbits.—Rabbits were immunized by single intravenous injection of killed pneumococci. The vaccine was prepared by suspending the sediment after centrifugation of an 18 hour Felton's broth culture of Type I pneumococcus in physiological saline solution to give a concentration of a billion cells per ml.

Testing for the presence of specific soluble substance in serum and exudates.—A modification of the method of Bukantz, de Gara and Bullowa (1942) was used. For the test 200 mouse units of antipneumococcic serum contained in 0.2 ml were added to 0.2 ml in 10×75 mm tubes varying dilutions of the material to be tested; the dilutions used were 1:5, 1:10, 1:15, 1:20, etc. After thorough mixing the tubes were centrifuged at 2,000 r.p.m. for 30 minutes. Reading was facilitated by holding the tubes against a blue light and looking through the bottom of the tube from above. The presence and amount of packed sediment in the form of a button on the bottom of the tube was noted and the tube showing maximum sediment was considered to be the one containing the optimum amount of soluble substance to

antibody used. The highest dilution showing this effect was used to denote the concentration of soluble substance.

The serum containing the precipitating antibody was obtained from 2 sources. One was a commercial preparation containing 5,000–10,000 mouse units per ml. The other was obtained from rabbits that had received a series of 3 immunizing doses of Type I pneumococcus vaccine. This serum was carefully standardized against the known serum before use. Before the tests were set up all the reagents used were clarified by centrifugation at 2,000 r.p.m. for 15 minutes.

Preparation of soluble antigen of pneumococcus.—Blood was collected from rabbits moribund from pneumococcal septicemia and allowed to stand overnight in the refrigerator. After removing the serum from the clot it was allowed to remain at room temperature for 8–10 hours, after which it was centrifuged, diluted 1:5, passed through Seitz filter and then heated at 56 C for 20 minutes; sterility tests were then made. Precipitin tests on this preparation revealed positive test at a dilution of 1:100,000.

Mice that died of pneumococcus infection also were the source of this material. A pool of the peritoneal washings of several mice was obtained. Then the heart, lungs and liver were removed, minced and placed in the peritoneal washings and this mixture allowed to extract over night in the icebox. After diluting with an equal volume of physiological saline solution the material was passed through a Seitz filter. Precipitin tests on this material were positive at 1:1,000,000.

These materials represent high concentrations of the active substance. This type of preparation was used in preference to soluble antigen prepared from pneumococcus culture because it represents an antigen more directly comparable to that formed during active infection.

EXPERIMENTAL

Comparative effect of penicillin and sulfonamide drugs on the clinical and antibody response of normal rabbits to pneumococcus infection.—Two groups of 20 rabbits each were infected as described. Treatment with penicillin or sulfapyridine was initiated at 4 or 24 hours after infection; treatment with sulfadiazine or sulfamerazine was initiated after 24 hours only.

The comparative clinical response to penicillin and sulfapyridine is shown in

figure 1. It is apparent that there was complete failure of the lesion to develop in rabbits in which penicillin therapy was initiated 4 hours after inoculation, but a more marked local lesion, a moderate systemic reaction as measured by

The effects of the 2 drugs on the outcome of the lesions were markedly different. In the 24 hour penicillin series the severity of the lesions as indicated in figure 1 was essentially unchanged after therapy was started. In several animals

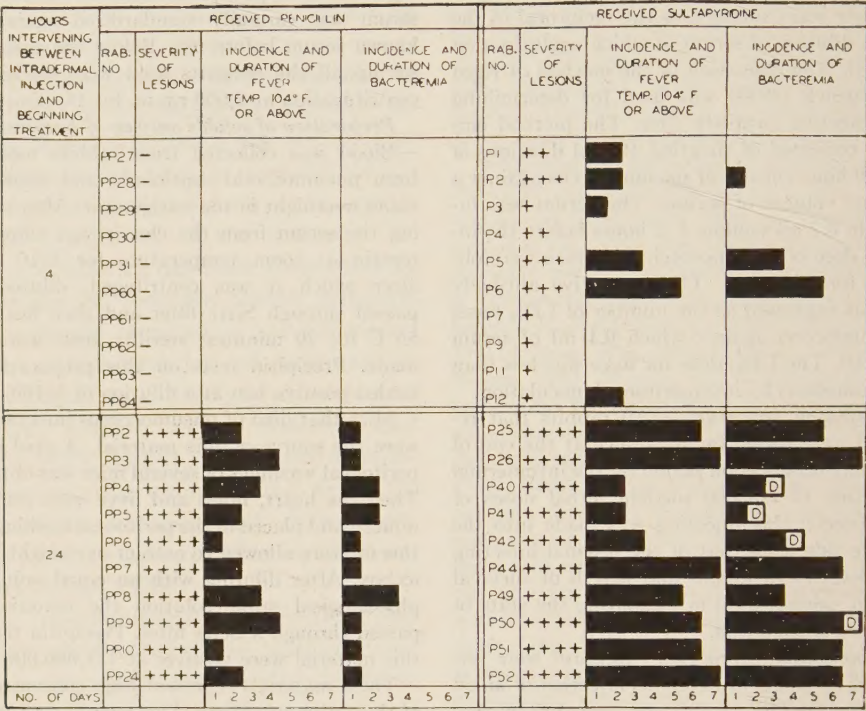


FIG. 1.—Clinical summary of intradermal pneumococcus infection of rabbits treated with sulfapyridine or penicillin.

Plus signs represent the severity of the lesions, from + to represent a tuberculin type lesion to ++++ to represent one of maximal intensity consisting of edema extending across the midline. Minus signs indicate that no lesions developed. The bars indicate the number of days during which fever (104 F or above) and bacteremia persisted. D indicates death of animal and is placed so as to indicate day of death.

temperature rise, and a transient bacteremia occurred when treatment was delayed until 24 hours after inoculation. This was in marked contrast to the response to sulfapyridine therapy; complete protection was not provided when therapy was initiated at 4 hours and when delayed 24 hours fever and bacteremia persisted for several days. Four rabbits in the 24 hour sulfapyridine series died.

regression of lesions occurred; only 2 progressed to necrosis. In contrast to this the lesions of all the rabbits in the 24 hour sulfapyridine series progressed to necrosis; none showed any evidence of regression.

The antibody response of these animals is summarized in figure 2; the series of animals in which penicillin therapy was initiated at 4 hours is omitted since no evidence of antibody response could

be detected. The 4 hour sulfapyridine series showed measurable protective antibody titers as early as 2 days, and the titer continued to rise throughout the period of observation. The agglutinin response was, however, very poor and antibody could not be detected before the 6th day and then only to minimal titers. In the 24 hour sulfapyridine series, however, both protective antibody and

Examination of lesion fluid was possible only from animals in the 24 hour series. No positive cultures were obtained from the 24 hour penicillin series 18–24 hours after therapy was initiated. This was in marked contrast to what was found in the sulfapyridine-treated rabbits; all fluids contained viable pneumococci for 4–5 days after the lesions became maximal. Specific soluble sub-

TABLE 1.—*Results of re-infection of rabbits recovered from intradermal pneumococcus infection treated with penicillin or sulfapyridine.*

Hours intervening between intradermal injection and beginning treatment	Received penicillin				Received sulfapyridine			
	Rabbit no.	Severity of original lesions	Severity of lesions after re-infection	Outcome	Rabbit no.	Severity of original lesions	Severity of lesions after re-infection	Outcome
4	PP27	—	++++	D5	P1	++	+	S
	PP28	—	++++	D4	P2	+++	++	S
	PP29	—	++++	D6	P3	+	+	S
	PP30	—	++++	D5	P4	++	++	S
	PP31	—	++++	D6	P5	+++	+++	S
	PP60	—	++++	D4	P6	+++	+++	S
	PP61	—	++++	D6	P7	+	+	S
	PP62	—	++++	D6	P9	+	++	S
	PP63	—	++++	D5	P11	+	++	S
	PP64	—	++++	D6	P12	+	+	S
	PP2	++++	—	S	P25	++++	—	S
	PP3	++++	—	S	P26	++++	+	S
24	PP4	++++	—	S	P40	++++D	—	S
	PP5	++++	—	S	P41	++++D	—	S
	PP6	++++	—	S	P42	++++D	—	S
	PP7	++++	—	S	P44	++++	+	S
	PP8	++++	—	S	P49	++++	+	S
	PP9	++++	+	S	P50	++++D	—	S
	PP10	++++	—	S	P51	++++	—	S
	PP24	++++	—	S	P52	++++	—	S

agglutinin responses were delayed and reduced in titer when they appeared. The maximum antibody response observed was in the 24 hour penicillin series; the protective antibody titer was markedly superior to that of the 24 hour sulfapyridine series. The agglutinin response did not, however, appear to parallel the protective antibody titer.

The results of re-infection are given in table 1. All animals in the 4 hour penicillin series died, although the lesions developed more slowly and death occurred after 5–6 days in contrast to control animals which died in 24–36 hours. None of the rabbits in the 24 hour penicillin series died and no rabbit in either of the sulfapyridine series died after re-infection.

stance was present in all fluids from both penicillin- and sulfapyridine-treated animals throughout the period of observation.

While the rabbits that received sulfadiazine and sulfamerazine appeared to eat better and to lose less weight during therapy, the other signs of infection differed very little from those of the 24-hour sulfapyridine series. No deaths occurred. The antibody response of these rabbits is summarized in figure 3. In general the antibody response appeared to be better than the 24 hour sulfapyridine series; however, it was much less than that of the 24 hour penicillin rabbits. Whereas in the 24 hour penicillin series protective antibody response showed a moderately sharp rise early in

the course of the infection to reach a titer of approximately 100,000 LD₅₀ at 12 days, that of the sulfadiazine and sulfamerazine series showed a markedly delayed response, and the antibody titer rose only to a level of 5,000–10,000 LD₅₀

antibody formation, (2) penicillin stimulates antibody production or (3) antibody formation is neither stimulated nor inhibited by these drugs but persistence of the infection attributable to less effective action of the drug, and cor-

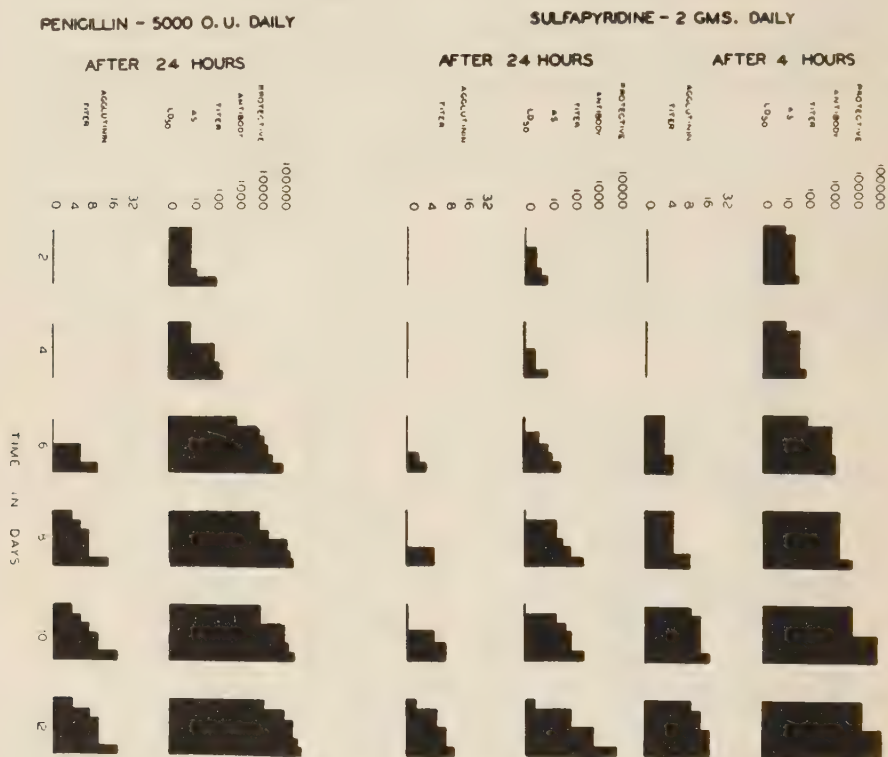


FIG. 2.—Antibody response of rabbits to intradermal pneumococcus infection treated with sulfapyridine or penicillin.

at the end of the observation period. The agglutinin response was very poor in both series. Moderately severe lesions, of (++) intensity, developed after re-infection but no deaths occurred.

These results show that in well established infections, as in the 24 hour series, penicillin-treated rabbits exhibit an enhanced antibody response while rabbits that receive sulfonamide drugs fail to develop high titer antibodies. There appear to be 3 more obvious possible explanations for these observations: (1) sulfonamide drugs inhibit

responding formation of fresh antigen. uses up formed antibody, resulting in reduced titers of circulating antibody. Comparison of the antibody response of the 4 hour sulfapyridine series with the 24 hour penicillin series suggests that the first is not tenable. In order to test the second possibility the following experiment was carried out.

Comparative effect of penicillin and sulfapyridine on the antibody response of normal rabbits to pneumococcus vaccine.—Three groups of 5 rabbits each were given approximately 1,000,000,000

killed pneumococci by intravenous injection. Five rabbits that served as controls received no drug. The other animals received either penicillin, 5,000 O.U. daily, or sulfapyridine, 2 g daily. Therapy was initiated immediately after injection of the vaccine and was continued for 4 days. Blood was collected on alternate days for protective antibody and agglutinin titrations and rabbits were tested for resistance as before.

Summary of the antibody response of rabbits to pneumococcus vaccine, without drug, and with penicillin or sulfapyridine, is given in figure 4. No significant difference was noted in the antibody response of these animals whether they received penicillin or sulfapyridine; moreover, these animals did not differ from those that received no drug. However, there was an appreciable difference between the response of rabbits that received pneumo-

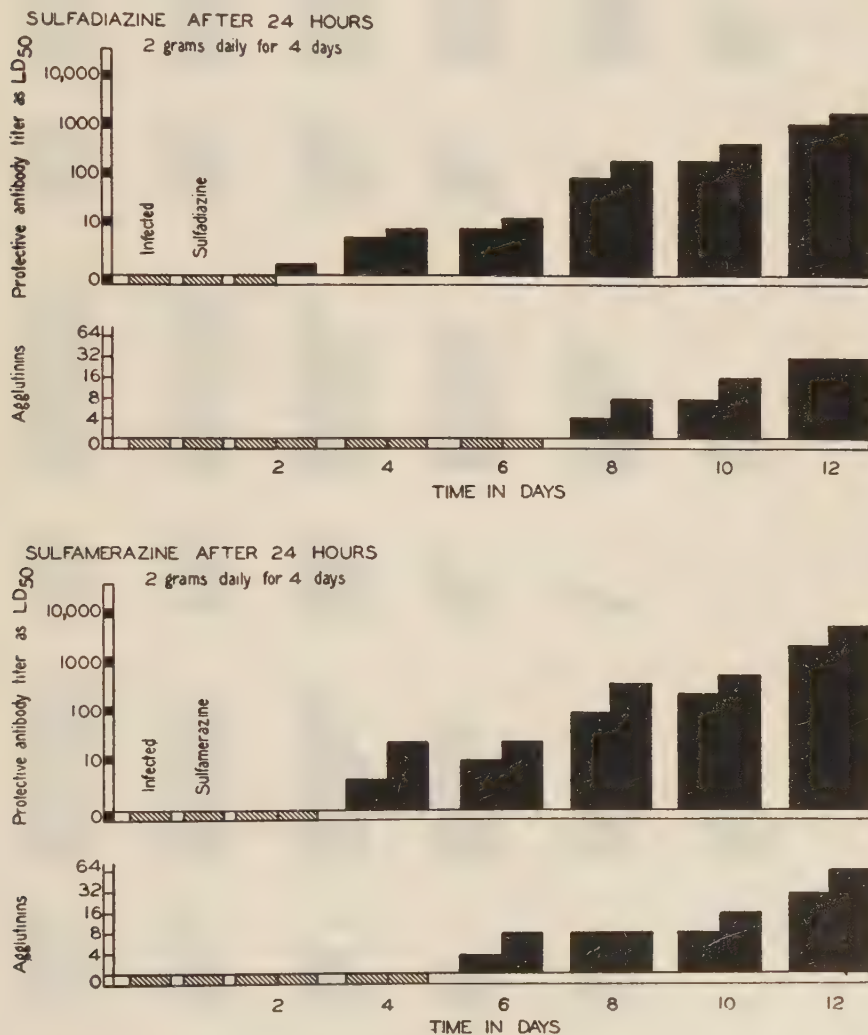


FIG. 3.—Summary of antibody response of rabbit to intradermal pneumococcus infection when therapy with sulfadiazine or sulfamerazine is initiated at 24 hours.

coccus vaccine and those which recovered from pneumococcus infection (fig. 2). Whereas in the vaccine animals the antibody titer, including both protective antibody and agglutinins, rose sharply during the early period of immunization but then hit a plateau, in general that of infected animals ex-

hibited a slight lag and the sharp rise occurred in the latter half of the observation period.

On re-infection none of the animals developed dermal lesions. These results are similar to those of the 24 hour penicillin series and the 4 hour sulfapyridine series (table 1) of the preceding experi-

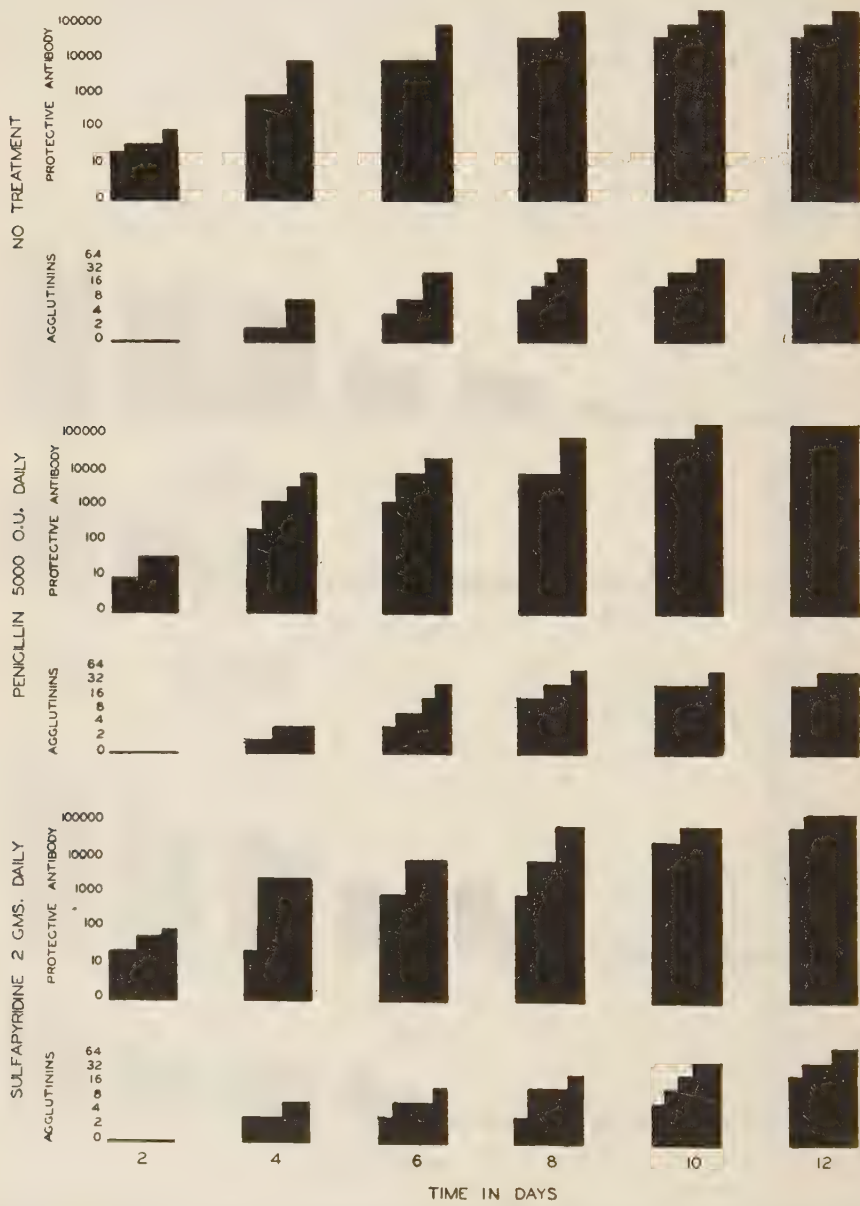


FIG. 4.—Summary of antibody response of rabbits to pneumococcus vaccine, without drug, and with penicillin or sulfapyridine.

ment. No deaths occurred among the animals in this series.

The results of the 2 preceding experiments seem to indicate that the sulfonamides and the antibiotic penicillin have no direct influence on the antibody response of infected animals or of rabbits immunized with killed pneumococci. Based on evidence available in these experiments it would appear that the reduced titers in the 24 hour sulfapyridine series is due to the formation of

the serum of rabbits with intradermal pneumococcus infection treated with penicillin or sulfonamide drugs.—Tests were performed on all serum specimens from the 4 and 24 hour penicillin and sulfapyridine series of rabbits and on all serums collected from rabbits in the sulfadiazine and sulfamerazine series reported in previous experiments. In addition serum specimens collected from 36 hour penicillin and sulfapyridine series described in a subsequent experi-

TABLE 2.—*Presence of specific soluble substance in serum from rabbits with intradermal pneumococcus infection treated with penicillin or sulfonamide drugs.*

Hours intervening between intradermal injection and initiation of therapy	Received penicillin										Received sulfapyridine									
	Time serum was collected in relation to intradermal injection (Highest dilution giving positive test)																			
	Serum no.	Be- fore	Days after								Serum no.	Be- fore	Days after							
			1	2	4	6	8	10	12	1			2	4	6	8	10	12		
4	PP27	0	0	0	0	0	0	0	0	P1	0	0	0	0	0	0	0	0	0	
	PP28	0	0	0	0	0	0	0	0	P2	0	0	0	0	0	0	0	0	0	
	PP29	0	0	0	0	0	0	0	0	P3	0	0	0	0	0	0	0	0	0	
	PP30	0	0	0	0	0	0	0	0	P4	0	0	0	0	0	0	0	0	0	
	PP31	0	0	0	0	0	0	0	0	P5	0	0	0	0	0	0	0	0	0	
	PP60	0	0	0	0	0	0	0	0	P6	0	0	0	0	0	0	0	0	0	
24	PP2	0	0	0	0	0	0	0	0	P25	0	0	1:5	0	0	0	0	0	0	
	PP3	0	0	0	0	0	0	0	0	P26	0	0	1:10	1:5	0	0	0	0	0	
	PP4	0	0	0	0	0	0	0	0	P44	0	0	1:20	1:20	1:20	1:10	1:10	0	0	
	PP5	0	0	0	0	0	0	0	0	P49	0	0	1:10	1:10	1:5	0	0	0	0	
	PP6	0	0	0	0	0	0	0	0	P51	0	0	1:5	0	0	0	0	0	0	
	PP7	0	0	0	0	0	0	0	0	P52	0	0	1:20	1:5	1:5	0	0	0	0	
36	PP25	0	0	1:10	1:5	1:5	1:5	0	0	P53	0	0	1:20	1:20	Died					
	PP26	0	0	1:20	1:10	1:5	0	0	0	P54	0	0	1:15	1:20	1:20	Died				
24	Received sulfadiazine										Received sulfamerazine									
	D1	0	0	1:5	1:5	0	0	0	0	M1	0	0	1:20	1:5	0	0	0	0	0	
	D2	0	0	1:15	1:5	0	0	0	0	M2	0	0	1:5	0	0	0	0	0	0	

fresh antigen during chemotherapy, and this neutralizes formed antibody. It seems reasonable to assume that the enhanced antibody response in the penicillin series is explainable on the basis of more effective therapeutic activity of penicillin which prevents the formation of fresh antigen. If these assumptions are correct then it should be possible to demonstrate the presence of soluble antigen of pneumococcus origin in the serum of inadequately treated animals; it should not be present in those adequately treated.

ment were tested for specific soluble substance.

The results are given in table 2. It is obvious from this table that none of the 4 or 24 hour penicillin series, and none of the 4 hour sulfapyridine series, showed a positive test for soluble substance. Also, none of the serums collected before infection, or 24 hours after infection, contained sufficient soluble substance to be detected by the method employed. When therapy was delayed longer than 24 hours, however, soluble substance in detectable amounts was formed, as evidenced by positive tests in the 36 hour

Presence of specific soluble substance in

penicillin and sulfapyridine series. Also this substance was detectable for 1 to 8 days in the serum of 6 of the 24 hour sulfapyridine series. Both sulfadiazine and sulfamerazine animals showed positive tests in their serums. It might be pointed out here that soluble substance was present in fairly high concentrations even in some serums that showed moderately high protective antibody titers.

These results suggest an apparent correlation between the presence of detectable soluble substance and a decreased antibody response; moreover, it is suggested that this substance, or other soluble antigens of pneumococcus growth, accounts for the delayed antibody response and reduced antibody titer of sulfapyridine-treated rabbits (cf. Blake, 1918; Downie, 1937b).

Dochez and Avery (1917), also Heidelberger and Avery (1923) showed that the soluble substance of pneumococcus origin is elaborated during growth of the organism either in the culture tube or in the human body, and Cole (1917) found that a large amount of soluble substance from pneumococcus growth is present in empyema fluids and in the blood of infected rabbits which possesses the power of neutralizing pneumococcus antibody. It seems possible in the present series of experiments that inadequate treatment, in the sense of incomplete control of the infection, allows the continuous production of antigen which neutralizes formed antibody; this in turn causes reduced titers of circulating antibodies. If this is correct then it should be possible to abolish measurable antibody by injecting excessive amounts of fresh antigen into presumably adequately treated animals during recovery from infection. The following experiment tests this effect.

Effect of injecting fresh antigen into

infected animals during the early period of treatment with penicillin.—The soluble antigen was obtained from rabbits moribund from pneumococcal septicemia as described in an earlier section. Infected animals were used as the source of this material because it was assumed that since pneumococci are readily lysed—autolysis after death—probably much of the antigenic stimulus in the infected animal is due to the presence of this type of antigen. Hence an effort was made to reproduce the conditions of infection in the animal.

For this experiment 4 rabbits were infected in the usual manner; 24 hours later penicillin therapy was initiated and this treatment continued for 4 days. Simultaneously with the drug 5 ml of serum containing the soluble antigen was injected intravenously every 3 hours 5 times daily during the first 2 days of therapy. Blood was collected daily for culture and on alternate days for antibody titrations. Tests for specific soluble substance in serum specimens were made. Protective antibody and agglutinin titrations and tests for resistance were carried out as before.

The blood cultures of all rabbits became negative within 24 hours after the initiation of therapy although no circulating antibody was detectable and tests for specific soluble substance showed this substance to be present in high concentration. The temperature remained elevated above 104 F for 4–5 days, but the animals continued to eat and no weight loss occurred.

The antibody response of these rabbits is summarized in figure 5. It is apparent that circulating antibody was completely abolished for the first 6 days, but then appeared as low titer agglutinin and barely measurable protective antibody. These titers rose slowly for the remainder of the observation period,

but they never reached a level greater than 1,000 LD₅₀. On re-infection all animals died in 4–5 days.

Avery and Morgan (1925), Avery and Heidelberger (1925), also Avery and Goebel (1933) and Downie (1937b) found the type specific polysaccharide of pneumococcus to be non-antigenic in rabbits and when present in relatively large amounts it could be demonstrated in the serum for several days after injec-

animals should manufacture soluble substance in quantities that would neutralize antibody, and if therapy could be initiated in time to save the animal, results similar to the previous experiment might be obtained. It was observed in other experiments that this substance did not begin to accumulate until after 24 hours. Hence, initiation of therapy was delayed 36 hours in the following experiment.

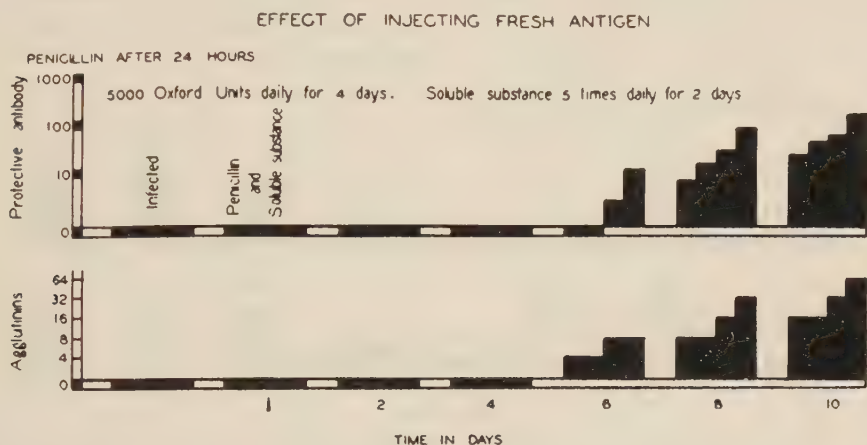


FIG. 5.—Effect of injecting fresh antigen into infected rabbits during the early period of treatment with penicillin.

tion. In the present series of experiments measurable antibody was abolished when known excess antigen was injected into penicillin-treated rabbits recovering from pneumococcus infection; several days were required for antibody to be formed in measurable amounts. It thus appears that excess soluble substance early in the course of the infection either inhibited antibody formation, suggested by death on re-infection, or, since it was slowly excreted, used up formed antibody. This same effect should be observed if soluble substance is allowed to accumulate in measurable quantity before drug therapy is initiated; in other words, after sufficient length of time growth of the organisms in the infected

Effect of prolonging the interval between intradermal injection and initiation of therapy on the amount of soluble substance in the serum and the clinical and antibody response of infected rabbits treated with penicillin or sulfapyridine.—Four rabbits were infected in the usual manner. Therapy was initiated 36 hours after infection. Two rabbits received penicillin and 2 sulfapyridine. The dosage of drugs was the same as in previous studies. Blood cultures and serological tests were carried out as before. After 12 days the surviving rabbits were re-infected to test for increased resistance.

The results of the tests for soluble substance are given in table 2. It is apparent that when therapy is delayed for

as long as 36 hours soluble substance is elaborated in detectable amounts which may persist even in spite of adequate therapy. The tests became negative during the period of observation in the penicillin-treated animals, but both rabbits receiving sulfapyridine died with high concentration of soluble substance detectable in the serum. Blood cultures from the penicillin-treated rabbits became negative in the usual length of time, 24–48 hours, but positive cultures

tive therapeutic effectiveness of penicillin and sulfapyridine by determining their ability to prevent production of antibody-neutralizing substances during the early phase of therapy; this property of the drug could be measured by determining the amount of injected antibody that is used up when administered after a given period of chemotherapy. The following experiment is concerned with this phase of the problem.

Effect of passive immunization on the

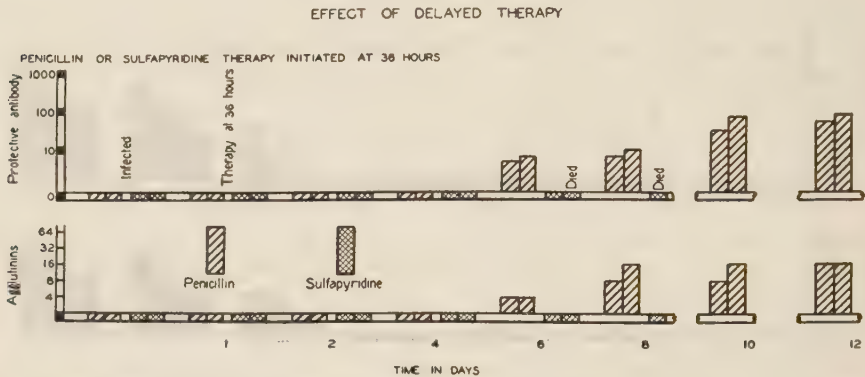


FIG. 6—Effect of prolonging the interval between intradermal injection and initiation of therapy on the antibody response of infected rabbits treated with penicillin or sulfapyridine.

were obtained from the blood of both rabbits receiving sulfapyridine until time of death.

The antibody response is shown in figure 6. No detectable antibody became apparent before the sixth day and then only to minimal titers, a response less in every respect than that of the 24 hour sulfapyridine series. Antibody titration on serum from the sulfapyridine animals collected immediately before death revealed no demonstrable antibody. The 2 penicillin-treated rabbits died on the 6th day after re-infection, with positive blood culture and lesions typical of non-immune animals.

Since infected serums and exudates contain antibody-neutralizing substances it seemed possible to obtain additional information as to the compara-

clinical and antibody response of rabbits with intradermal pneumococcus infection treated with penicillin or sulfapyridine.—

Two groups of 4 rabbits each were infected in the usual manner. Therapy with penicillin or sulfapyridine in doses identical with those of previous studies was initiated 24 hours after infection. After 24 hours of chemotherapy blood was collected for culture and 50 units of antipneumococcic serum (rabbit) per kg of body weight were given intravenously. Six hours later a second sample of blood was collected for culture and antibody titrations. This procedure was followed daily for 3 days. Chemotherapy was continued for 4 days and observations made for 10 days.

Simultaneously several normal rabbits were given 50 units of antiserum per

kg of body weight daily for 3 days and blood collected at the same intervals as those from the infected rabbits. Antibody titrations were carried out along with those of the infected rabbits. Only protective antibody titers were determined in this study.

The clinical course of the disease in the 2 groups of rabbits was similar after serum therapy was initiated. Animals ate well and no weight loss occurred. The lesions failed to go on to necrosis as was observed in untreated animals and in the 24 hour sulfapyridine rabbits, but began to regress after the first dose of immune serum. Lesion cultures in the penicillin treated rabbits became negative in 12–18 hours after penicillin therapy was initiated; but in the sulfapyridine series the cultures did not become negative until 48–72 hours after the first injection of immune serum. Soluble substance was present in all fluids of all animals of both series for 48 hours, the last time this material was available. The temperature of the penicillin-treated rabbits became normal in 24–48 hours and that of the sulfapyridine-treated animals in 24 hours after the first dose of immune serum. All rabbits were bacteremic at the time chemotherapy was begun; within 12 hours the blood cultures of the penicillin rabbits were negative, but the sulfapyridine series continued to be positive until 12 hours after the first dose of immune serum.

The results of passive immunization experiments are given in figure 7. The antibody titers of the 2 control rabbits showing the least and the greatest antibody response are placed alongside those of the 4 infected rabbits for comparative purposes. No reduction in the antibody titer of serum from the penicillin-treated rabbits occurred after the first injection of immune serum, as judged from the control animals. In other words, the titers fell within the

range of those of the normal controls. By the third day the antibody titers rose to a higher level than the highest control in 3 and equal to this control in 1. This additive effect was observed throughout the remainder of the observation period, the antibody titer steadily rising to reach a maximum of approximately 100,000 LD₅₀ at the 10th day.

These events were in contrast to those that occurred in the sulfapyridine series. There was a partial neutralization of injected antibody for the first 4 days in all infected rabbits. This neutralizing effect is indicated by differences in antibody titers of the infected rabbits as compared with the lowest response in the controls. The magnitude of these differences is unfortunately minimized by the necessity of using a logarithmic scale.

Because of the minimizing effect of this graph, the results are presented as fold-differences over the controls in figure 8. In this graph the neutralizing effect is represented by graphs that lie below the base line, and the additive effect, or active immunity, is represented by graphs above the base line. This line represents the range of passively immunized normal rabbits. It is apparent from figure 8 that no neutralization of injected antibody occurred in the penicillin-treated rabbits; rather, an additive effect soon became apparent. In the sulfapyridine-treated rabbits, on the other hand, a neutralization of injected antibody occurred, and only after the third injection of therapeutic serum was the antibody response of these rabbits greater than that of the controls.

Although soluble substance of pneumococcus origin does not appear to be antigenic for the rabbit, several workers (Schiemann and Casper, 1927; Saito and Ulrich, 1928; Schiemann, 1929; Wadsworth and Brown, 1931; Zozaya, 1932; Zozaya and Clark, 1933; Avery

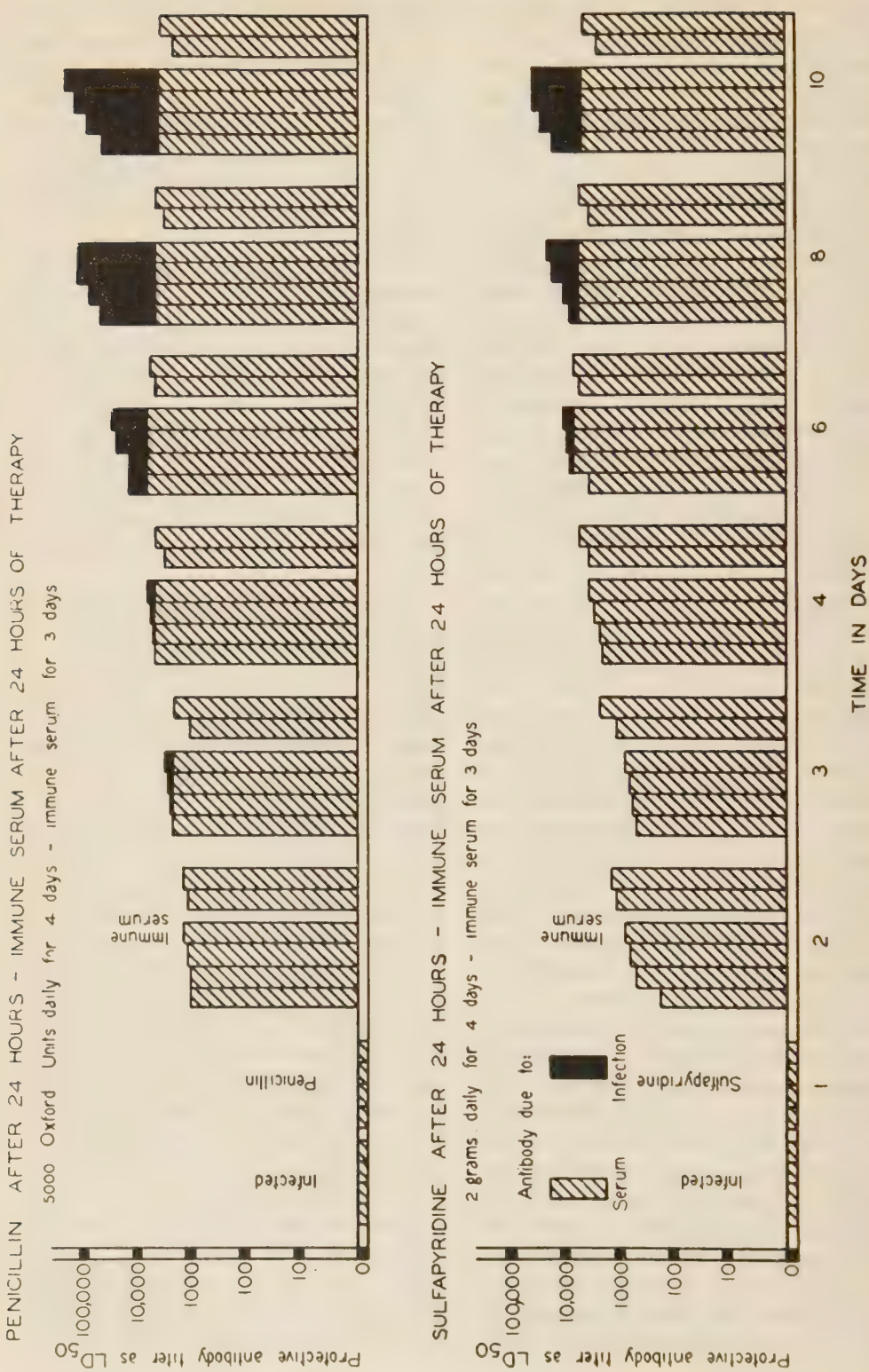


FIG. 7.—Effect of passive immunization on the antibody response of rabbits with intradermal pneumococcus infection treated with penicillin or sulfapyridine.

and Goebel, 1933; Felton, 1934; Downie 1937a) have shown that the material is antigenic for mice when administered in small but not in large doses. Based on the observations of these workers it seemed possible to test the validity of the interpretation of results obtained in the present study by comparing the effect of penicillin and sulfonamide drugs on the immune response of mice with pneumococcus infection when therapy was initiated at varying intervals after infection. Since the material in suitable doses is antigenic in mice to the extent they are made immune, and in larger doses it leads to no demonstrable immunity, it seemed this approach might give additional information on the general effect of chemotherapeutic agents on the immune response.

Of equal importance is the comparative effect of penicillin and the sulfonamides on development of immunity to infections when therapy is initiated early. In the 4 hour penicillin series it was observed that no demonstrable antibodies developed and death of the animals occurred after re-infection. It seemed important to know the length of time required to manufacture sufficient antigen to induce immunity, without at the same time allowing too much antigen to accumulate to depress the immune response. The next experiment is concerned with the quantitative aspects of this problem.

Comparative effect of penicillin and sulfadiazine on development of immunity of mice to pneumococcus infection when therapy is delayed.—Mice weighing approximately 20 g were infected by the intraperitoneal inoculation of 50 median lethal doses (mice) of Type I pneumococcus. At 0, 2, 4, 6, 8, 10, 12, 18 and 24 hours following infection therapy with penicillin or sulfadiazine was initiated. Penicillin was given in 5 subcutaneous injections of 30 O.U. each per

day, and sulfadiazine was administered intragastrically in 20 mg doses twice daily. Therapy was carried out for 3 days and observations made for 6 days. At the end of the observation period surviving mice were inoculated intraperitoneally with 10-fold dilutions of the challenging dose of pneumococci. The level of immunity was expressed as the dose causing 50% mortality.

The results are summarized in figure 9. Deaths are recorded as those occurring during therapy and as those occurring after therapy was discontinued. The average survival time of mice during the post-treatment period was computed according to the method of Whitby (1937). Examination of figure 9 shows significant differences in the number and time of deaths occurring in the treatment groups. No deaths occurred during therapy in the penicillin series when therapy was initiated within 10 hours after infection. A few deaths occurred in the 12 and 18 hour treatment groups, and 13% of the mice died during treatment when delayed for 24 hours. In the post-treatment period several deaths occurred in the 4, 6, and 8 hour treatment periods, but most of the late deaths in the penicillin series occurred when therapy was delayed 24 hours. The degree of immunity of the surviving mice is shown in column 6. Only a moderate increase in immunity developed in mice in which therapy was initiated earlier than 8 hours after infection. The maximum resistance was reached in the 12 and 18 hour treatment groups, but appeared reduced in the 24 hour penicillin mice.

These results are in marked contrast to those in the sulfadiazine series. Many early and late deaths occurred in the early treatment groups, and the number of deaths progressively increased as therapy was delayed. There was a progressive fall in the average time of sur-

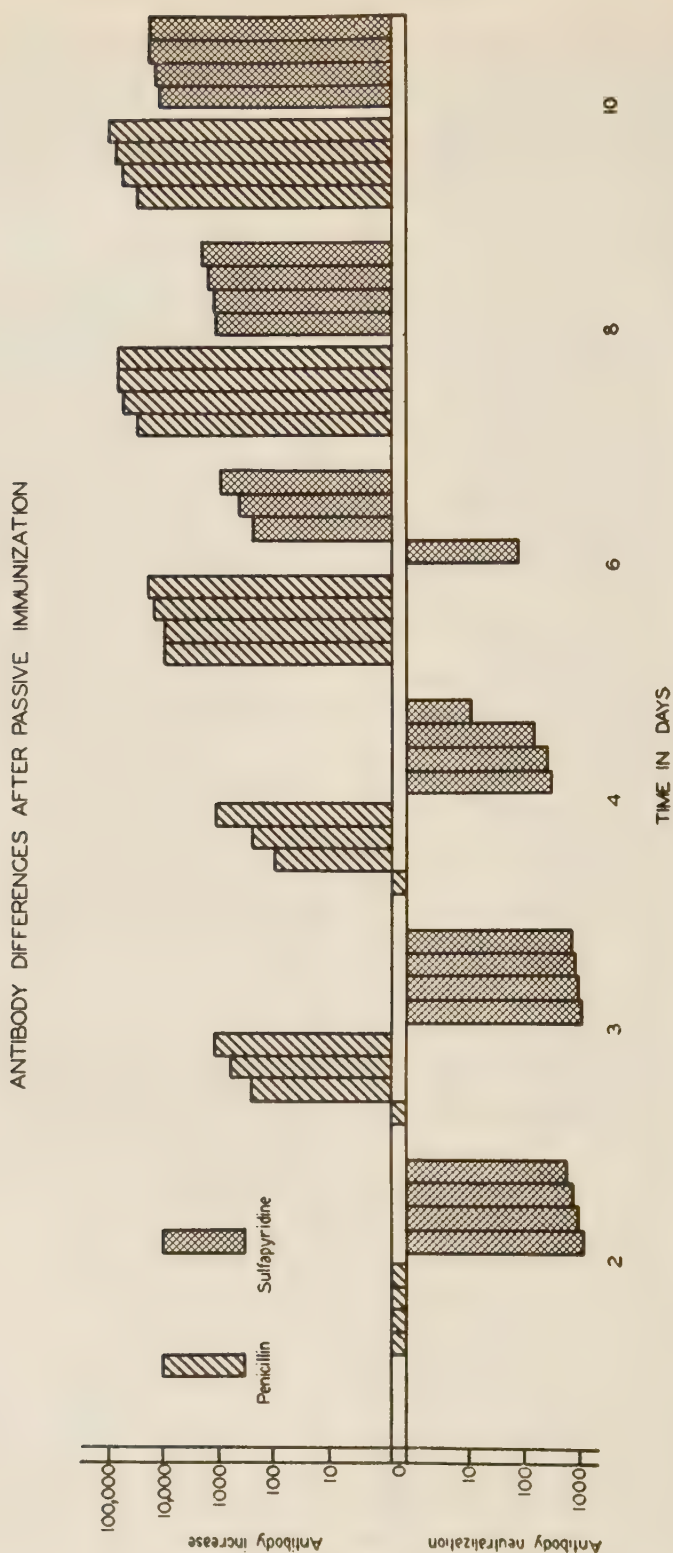


FIG. 8.—Antibody differences of passively immunized infected rabbits treated with penicillin or sulfapyridine.

vival during the post-treatment period and a corresponding decrease in the level of immunity of survivors. Whereas in the penicillin series the greatest immune response occurred relatively late, in the sulfadiazine mice the greatest response occurred early and declined as therapy was delayed. At 24 hours both the sulfadiazine and the penicillin mice exhibited about the same level of immunity.

Comparative effect of soluble antigen on the therapeutic and immune response of infected mice treated with penicillin or sulfadiazine.—In the preceding experiment it was noted that when therapy was delayed the immune response was markedly depressed. It was assumed

previously described, intraperitoneally every 3 hours for 6 doses, in addition to penicillin or sulfadiazine. Therapy was initiated immediately after infection and continued for 2 days. Observations were made for 6 days, after which the level of immunity of survivors was determined.

The results are given in table 3. It is apparent that the presence of excess antigen had little effect on the therapeutic response to penicillin. On the other hand, sulfadiazine, in the presence of soluble substance and corresponding lowered immunity, was unable to eradicate the infection as evidenced by several late deaths. The presence of this

TABLE 3.—*Effect of pneumococcus soluble antigen on the therapeutic and immune response of infected mice treated with penicillin or sulfadiazine.*

Amount of soluble substance inoculated	Received penicillin			Received sulfadiazine		
	Deaths		Level of immunity as LD ₅₀	Deaths		Level of immunity as LD ₅₀
	During therapy	After therapy		During therapy	After therapy	
3 ml	0	0	8	2	8	15
0	0	0	58	1	0	260,000

that excess soluble substance (excess in the sense that free polysaccharide existed in addition to that bound to the cells) might be responsible for this effect. In order to test this assumption excess antigen was injected simultaneously with the drugs during treatment of intraabdominal infections in mice. This approach also seemed to afford a method of determining the relative importance of demonstrable immunity to successful chemotherapy with penicillin and sulfadiazine.

For this experiment 4 groups of 30 mice each were infected by the intraperitoneal inoculation of approximately 5,000 median lethal doses of pneumococci. Two groups received only penicillin and sulfadiazine as before; the other 2 groups received 0.5 ml of soluble antigen, prepared from infected mice as

substance in the amounts injected appeared to mask the immune response by using up antibody by combining with it so that it was not available for combating the infection. These results are in part supported by the observations of Spring, Lowell and Finland (1940) that culture filtrates of pneumococcus exerted a neutralizing effect on the action of immune serum even in the presence of sulfapyridine.

A marked difference was noted between the penicillin- and sulfadiazine-treated mice that received no soluble substance. No immunity resulted from the infection when penicillin was used in treatment, but an effective immune response was induced in the sulfadiazine treated mice. These results are in agreement with those of the preceding experiments; the infecting dose in this experi-

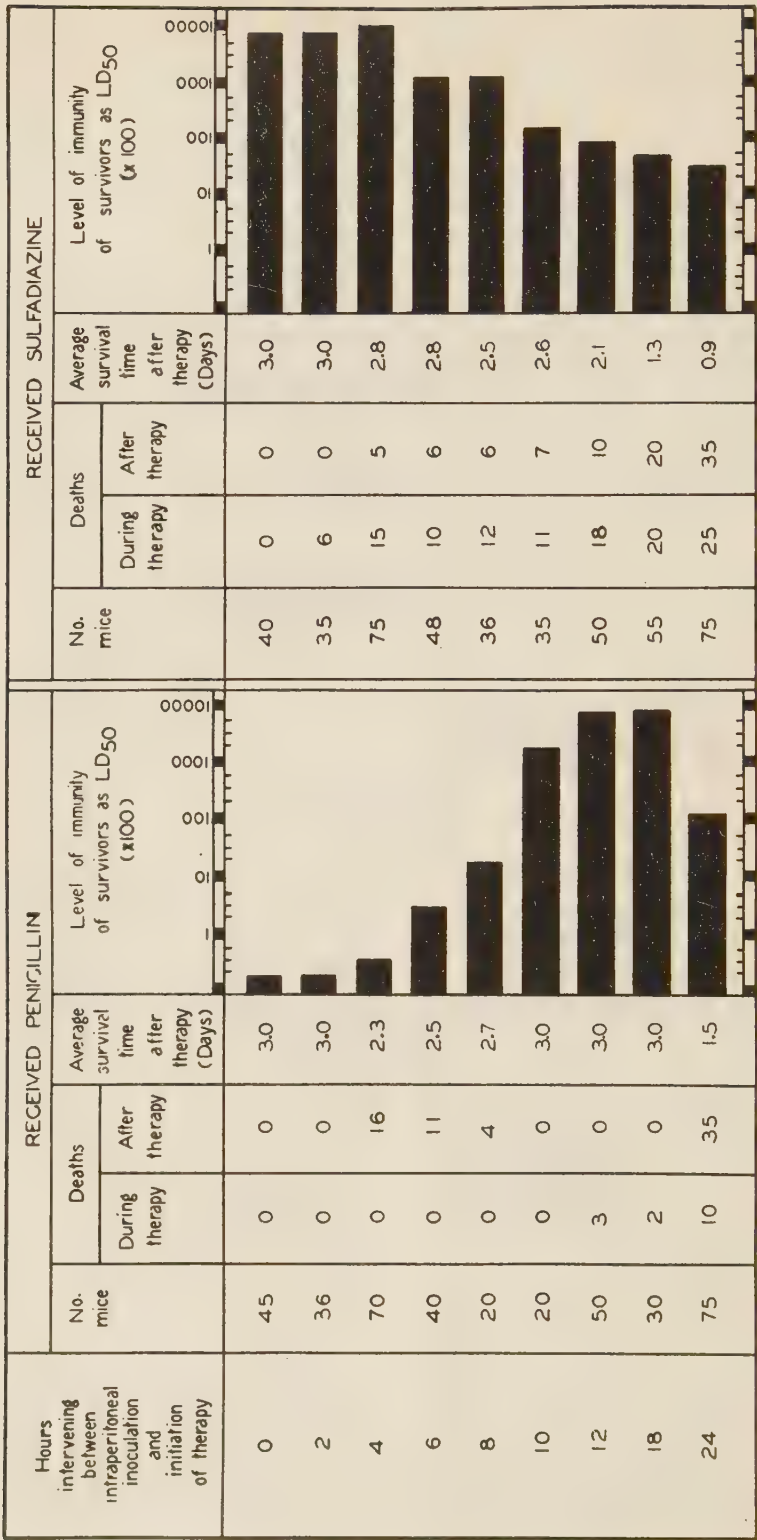


Fig. 9.—Comparative effect of penicillin and sulfadiazine on development of immunity of mice to pneumococcus when therapy is delayed.

ment apparently was not sufficient to induce an immune response, and penicillin quickly stopped cell multiplication before more antigen was formed. Because of the lag in sulfadiazine activity, or the incomplete bacteriostasis of this drug, sufficient antigen was formed during therapy to stimulate an effective immunity.

DISCUSSION

The marked therapeutic superiority of penicillin over the sulfonamide drugs noted by others in both experimental and clinical studies is apparent here. The differences in the chemotherapeutic response to the 2 types of drugs appear to be accounted for on the basis of incomplete bacteriostasis and lag in activity of the sulfonamide drugs; also penicillin does not appear to be affected by infected serums and exudates as evidenced by the rapid regression of the lesions and disappearance of viable pneumococci from lesion material from infected rabbits. This is in agreement with the observations of Abraham and associates (1941) and of Dawson, Hobby, Meyer and Chaffee (1943).

Failure of the lesions to develop in the 4 hour penicillin series suggests that penicillin in conjunction with natural cellular and humoral activity is sufficient to eliminate the infection almost immediately; sulfonamides, on the other hand, do not appear to be able to provide sufficient protection to enable natural immunity to eradicate the infection.

The complete lack of antibody response of rabbits and the failure of development of demonstrable immunity in mice when penicillin therapy was initiated early are curious; possibly they are to be accounted for on the assumption that natural immunity in conjunction with the drug eliminated the infection before antibody response was in-

duced. Apparently penicillin, in contrast to the sulfonamides, is able to stop cell division immediately after contact with the organism. If one assumes the lag phase in vivo to be only 2 hours, actually it may be much more, theoretically there should be less than 200,000 pneumococci present at 4 hours when an inoculum of 10,000 cells is used to initiate an infection. Since blood cultures of rabbits were not positive until 8-10 hours after infection it is unlikely that generalization of the infection occurred before therapy was initiated; thus, insufficient antigen was produced to induce immunity (cf. Topley and Wilson, 1936). Although pneumococcus antigen when injected into the skin of a rabbit in small doses fails to induce type specific antibodies in the blood (Julianelle, 1930a), an increased resistance to both the homologous and heterologous types of pneumococci results (Julianelle, 1930b). Perhaps this explains in part the enhanced resistance of these rabbits in the absence of circulating antibodies.

Presumably a delay of 24 hours before initiation of therapy allows multiplication of pneumococci to the extent that an adequate antigenic stimulus is provided. This appears to be indicated by the satisfactory antibody response of rabbits in the 24 hour penicillin series (fig. 2) and the immune response of mice treated after 10-12 hours (fig. 9). In general the elaboration of demonstrable antibodies in the rabbit and the development of immunity in mice appear to parallel progress of recovery. Comparison of antibody response of infected rabbits treated with sulfapyridine or penicillin with the clinical data summarized in figure 1 shows that the clinical response is associated with protective antibody titer; agglutinin titer does not appear to be a reliable index of an effective immune response. The development of a high level of immunity

in the 4 hour sulfapyridine series is consistent with the therapeutic activity of this drug. The lag in activity and incomplete bacteriostasis allow multiplication of pneumococci so that a suitable amount of antigen is formed; subsequently, because the drug is started early, the infection is held in check and an optimum antigen-antibody ratio is maintained. Under these conditions drug-induced bacteriostasis in conjunction with acquired immunity is followed by a good therapeutic response.

On the other hand, persistence of the infection, and therefore antigenic stimulus, as indicated especially by the 24 hour sulfapyridine series, is not accompanied by the development of high titer protective antibody. The more obvious possible explanations are: (1) penicillin stimulates antibody production, (2) sulfapyridine inhibits antibody formation, or (3) antibody formation is neither stimulated nor inhibited by these drugs but persistence of the infection attributable to less effective action of sulfapyridine, and corresponding formation of fresh antigen, uses up formed antibody, resulting in reduced titers of circulating antibody. That the first possibility is unlikely is indicated by failure of penicillin to enhance antibody formation in rabbits immunized with killed pneumococci (fig. 4). Comparison of the antibody response of the 4 hour sulfapyridine series with the 24 hour penicillin series, together with the results of the vaccine studies and the similarity of the antibody response following sulfadiazine and sulfamerazine therapy (fig. 3) to that following sulfapyridine therapy, suggests that the second is not tenable. Based on evidence available in these reports the third possibility seems the most probable.

The differences in type of antibody response of rabbits that received vaccine and those that recovered from infection

may be partly accounted for on the assumption that the killed pneumococci provide an adequate antigenic stimulus but do not produce fresh antigen subsequently to use up formed antibody. Almost all formed antibody is present at the time of antibody titration. Measurable antibodies thus accumulate and a sharp rise is observed in the early part of the immunization period. In contrast to this, live antigen present in infections even with the most efficient bacteriostasis, removes or neutralizes part of formed antibody during the early phase of chemotherapy, and a lag is observed in the curve of antibody response.

It is possible also that a qualitatively different immune response is induced in rabbits recovering from infections as compared with that of animals artificially immunized with vaccine; this in part might explain the different pattern of immune response, as evidenced by antibody response, in infected rabbits as compared with immunized ones. Campbell (1938) described "non-absorbable" antibody which became detectable late in infections, and not at all in artificially immunized animals, that was considered to be due to antigens of actively metabolizing cells but not present in quiescent or killed cells. The delay in the formation of this type of antibody was thought to depend upon the length of time required for the elaboration of sufficient amount of antigenic substance to induce its formation. Although the relation of Campbell's observations to the results reported in the present communication may not be altogether clear, it is not unlikely that in pneumococcus infections such as reported here a non-absorbable type of antibody becomes functional, although late, in both protection and resistance tests.

It is possible also that a reproduction-inhibiting antibody participates in the

immune reactions that accompany recovery following chemotherapy of pneumococcus infection; this might account for some of the observed differences in detectable antibody and resistance of infected as compared with vaccinated rabbits. Although a reproduction-inhibiting antibody for bacteria has not been conclusively demonstrated, the work of Dochez and Avery (1916) suggests such a function for antipneumococcus serum, and Taliaferro (1932) has shown that a reproduction-inhibiting antibody develops in response to invasion with trypanosomes. It seems possible that this type of antibody, or a similar one, the nature of which is at present unknown, may be elaborated in animals in response to infections treated with chemotherapeutic agents. Owing to the masking effect of excess antigen in the sulfonamide-treated animals it is not as apparent as in the penicillin-treated ones; when the animals are artificially immunized with killed antigen this difference is not manifested. The delayed deaths, along with the delay in development of dermal lesions and prolonged survival time after reinfection of the presumably non-immune 4 hour penicillin rabbits might be partly accounted for on the basis of such an antibody.

Soluble substances of pneumococcus growth are known to be present in large amounts in the blood and tissues of infected animals (Dochez and Avery, 1917; Heidelberger and Avery, 1923). Pneumococcus soluble substance does not induce protective antibodies or agglutinins when injected into the rabbit (Avery and Morgan, 1925; Avery and Heidelberger, 1925; Downie, 1937b) but this haptenic substance possesses the power of neutralizing antibody (Cole, 1917; Felton and Bailey, 1926; Sia, 1926; Wadsworth and Brown, 1927; Sickles, 1927; Ward, 1930, 1932) and of decreas-

ing the functional activity of the defense mechanism of infected animals (Felton and Bailey, 1926; Bigg, 1941). Day (1945) has recently described an anti-immunity or "opposition" factor, isolated from pneumococci, which appears to lessen the development of type specific immunity. It is possible that the soluble substance and anti-immunity factor function to neutralize formed antibody. Even with adequate antigenic stimulus, which undoubtedly is available when therapy is delayed, an enhanced immune response is not always observed unless chemotherapeutic action maintains an optimum antigen-antibody relationship. It is possible that penicillin quantitatively reduces the amount of living antigen and its soluble products through its greater bacteriostatic activity; sulfonamide drugs, on the other hand, being less active, are unable to completely stop growth, so that antibody-neutralizing or inhibiting substances continue to be formed. The time at which therapy is initiated, however, influences the outcome of chemotherapy in this respect. Results of tests for soluble substance in serum from infected rabbits (table 2) indicate that the lapse of at least 36 hours is necessary for the substance to accumulate in measurable amounts under these experimental conditions.

The presence of the material in the 24 hour sulfapyridine series of rabbits would appear to indicate that multiplication of pneumococci and subsequent elaboration of haptenic substance occurred during sulfapyridine therapy; its absence in the corresponding penicillin series suggests that this drug becomes therapeutically effective before the material is produced in appreciable amounts. These events appear to be important to the outcome of chemotherapy for the appearance of demonstrable antibodies seems to parallel progress of re-

covery regardless of the type of chemotherapy. In the case of penicillin, however, immunity appears to function merely as an extra safeguard in chemotherapeutic activity.

The effect of soluble substance on the immune response of rabbits is indicated by the results of the 36 hour penicillin and sulfapyridine series. Tests for this substance at the time therapy was initiated indicated its presence in fairly high concentration. A corresponding depression of the antibody response was observed; rabbits that were re-infected later died of lesions typical of non-immune animals. This suggests that when the material is allowed to be elaborated in measurable quantities its effect is to lower the antibody titer; this seems to be effective for several days, at least until the substance is eliminated or neutralized by antibody. This same effect was observed when known excess amounts of antibody-neutralizing substance was injected into infected rabbits treated with penicillin.

The results of passive immunity experiments bear more directly on this problem. In penicillin-treated rabbits no measurable antibody-neutralizing effect was obtained after the injection of a known amount of antibody; part of this antibody was neutralized in the sulfapyridine-treated rabbits. Since soluble substance was present in the serum of sulfapyridine-treated rabbits and not in the penicillin-treated ones when antibody was injected, it seems reasonable to assume that this substance was responsible for the antibody-neutralizing effect.

The finding of soluble substance in serums that possessed measurable protective antibody is curious inasmuch as Downie (1937b) and others have found that antibody and the corresponding polysaccharide were never found together in any sample of serum. Perhaps

this may represent a zone phenomenon occurring *in vivo* (Ward, 1930; Coventry, 1927). On the other hand, it may be due to the presence in the serum of protective antibody not neutralized by homologous specific soluble substance (Ward, 1932; Sabin, 1931). Whatever the explanation, it may be assumed that the conditions that allowed the elaboration of this substance would not favor development of excess, measurable antibody.

While in general the results of the mouse experiments confirm and extend the findings in rabbits, certain aspects of these experiments seem difficult to explain. When started immediately, or soon after, the initiation of infection with a small inoculum penicillin eradicated the infection in the absence of a demonstrable immune response, as evidenced by lack of late deaths. However, when therapy was delayed 4-8 hours only a very slight immunity developed and a number of late deaths occurred; yet when therapy was delayed 10-18 hours no late deaths occurred and a good immune response resulted. With sulfadiazine, on the other hand, the best immunity developed when therapy was initiated early; as the interval between infection and initiation of therapy was extended the number of late deaths steadily increased and the level of immunity fell. After 24 hours both the penicillin- and sulfadiazine-treated mice exhibited a lowered immunity and a large number of late deaths occurred in each group.

It is possible these observations are explainable on differences in chemotherapeutic activity of drug and size of dose of antigen present during the infection. The antibiotic penicillin is able to control quickly the infection so that if it is started early the antigenic stimulus is abolished. But if it started early, before local abscesses form, penicillin in

conjunction with natural immunity eliminates the organism completely. Although this drug appears to exert a bactericidal effect *in vitro*, it has been demonstrated that usually not all the organisms are killed; this feature of penicillin activity appears even more definite *in vivo*. When penicillin is delayed only sufficiently long to allow localization of infecting microorganisms, but not long enough to allow formation of adequate antigen for immune response, several late deaths occur. Presumably these late deaths are due to latent infections (cf. Chain and Duthie, 1945); with an effective immunity these relapses are prevented.

When the time between infection and initiation of therapy, either with penicillin or sulfadiazine, was prolonged the average survival time in the post-treatment period and the immunity of survivors decreased. The lowered immunity and high number of late deaths are curious; apparently developing immunity in conjunction with drug is insufficient to control and eradicate the infection. Results of the experiments in which excess antigen was injected into infected mice suggest that this material in large amounts prevents development of effective immunity. Actually an accelerated immune response might be expected because of the additional antigenic stimulus but this would not necessarily be true because the additional antigen combined with that in the pneumococci might add up to an overwhelming dose and thus inhibit the immune response. This observation appears to be in agreement with the findings of others (Schiemann and Casper, 1927; Saito and Ulrich, 1928; Schiemann, 1929; Zozaya, 1932; Zozaya and Clark, 1933; Avery and Goebel, 1933; Felton, 1934; Downie, 1937a).

It would appear from the results of these studies that successful therapy

with penicillin occurs relatively independently of antibody formation. However, the participation of some type of immune mechanism cannot be excluded. To assume that there is lack of time for functional immunity, either natural or acquired, to be effective in penicillin therapy is hardly justifiable; even acquired immunity may become active in a matter of a few hours, although this may be difficult to demonstrate. In spite of lack of demonstrable antibodies in infected rabbits that were treated early, there was some evidence of increased resistance on re-infection. It thus seems reasonable to assume that acquired immunity was functional in the observed response to drug therapy. It is possible of course, that this cooperation is not necessary for successful penicillin therapy. On the other hand, when the infecting bacteria become established in the tissues, without provoking immunity, penicillin does not appear to be able to completely eliminate the organism. Under these conditions immunity would appear to be helpful.

These studies also indicate that measurable circulating antibody is not essential to successful penicillin therapy, for when protective antibodies were abolished by injecting neutralizing antigen, blood cultures became negative in the usual length of time. No relapses occurred in these rabbits after cessation of drug. However, an immune mechanism, perhaps even acquired immunity, cannot be excluded. It is possible that circulating antigen induced a type of local immune response in large organs such as the liver and spleen (cf. Taliaferro, 1934) in the absence of excess circulating antibody. This type of local immunity would function to prevent relapse, but would not be sufficient to protect against an overwhelming infection such as occurs after re-infection.

The differences in the type of chemo-

therapeutic response observed in penicillin and sulfonamide therapy appear to depend on the comparative bacteriostatic activity of the 2 drugs. In the case of the sulfonamide drugs bacteriostasis is slow and incomplete, and their effectiveness varies directly with the concentration of drug and inversely with the size of the original population (Whitby, 1938; Chandler and Janeway, 1939; McIntosh and Whitby, 1939; Spring, Lowell and Finland, 1940; Lowell, Strauss and Finland, 1941). It thus becomes necessary for the immune mechanism to function as an adjuvant to sulfonamide chemotherapy. The chemotherapeutic response parallels the antibody response and is dependent on it for control of the infection.

SUMMARY

A study was made of the comparative effect of the antibiotic penicillin and the sulfonamide drugs, sulfapyridine, sulfadiazine and sulfamerazine on the clinical and immune response of rabbits and mice to pneumococcus infection. Also an attempt was made to indicate how immunity functions in bacterial chemotherapy.

When therapy was initiated 4 hours after intradermal inoculation of Type I pneumococcus in rabbits, penicillin prevented the development of the dermal lesion and no demonstrable circulating antibody was produced. Sulfapyridine therapy, on the other hand, did not prevent development of the local lesion, or systemic disease, and recovery from the infection was followed by high titer antibody response. As the interval between infection and initiation of therapy was increased a reduced antibody response occurred in rabbits that were treated with the sulfonamide drugs. In contrast to this, rabbits that received penicillin exhibited a good immune re-

sponse, as evidenced by high titer protective antibody and agglutinins.

Based on evidence presented in this series of experiments the type of antibody response of infected rabbits treated with penicillin or sulfonamide drugs appears to be determined by the therapeutic efficacy of the drugs used. When started soon after infection, penicillin, in conjunction with natural immunity, is able to eliminate the infection before antibody response is induced. Incomplete bacteriostasis and the lag in activity of sulfonamide drugs, on the other hand, allow multiplication of pneumococci so that a suitable amount of antigen is formed after initiation of therapy; subsequently, because the drug is started early, the infection is held in check and an optimum antigen-antibody balance is maintained. Under these conditions sulfonamide-induced bacteriostasis is followed by a high titer antibody response. When initiation of therapy is delayed, penicillin appears to be able to quickly control the infection so that excess antigen in the form of cells and soluble substance is not formed and high titer antibody response is obtained. The less therapeutically active sulfonamide drugs appear to allow the infection to persist, as evidenced by positive blood cultures, with corresponding formation of fresh antigen which uses up formed antibody, resulting in reduced titer of circulating antibody.

The effect of soluble substance of pneumococcus growth on the chemotherapeutic response of infected rabbits and mice treated with sulfonamide drugs and penicillin was studied. When the immune response of rabbits and mice was abolished by the presence of measurable excess soluble antigen no difference was noted in the therapeutic response following penicillin therapy as compared with that of animals with

high titer antibody concentration. A poor therapeutic response followed sulfonamide therapy under the same conditions.

While successful penicillin therapy appears to be relatively independent of an immune mechanism, the participation of some type of immunity cannot be excluded; it appears, however, that the immune mechanism is much less important to penicillin therapy than to sulfonamide therapy.

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